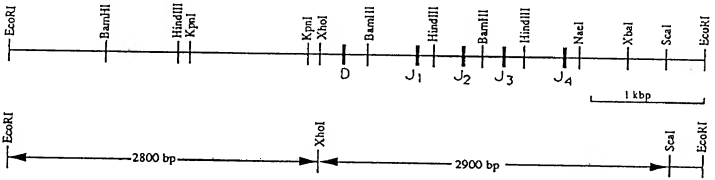
Ziska
800/002

Mouse Heavy Chain J Genes Inactivation Vector

(A) Targeted mouse heavy chain J genes



(B) Inactivation vector mDΔJ.Neo

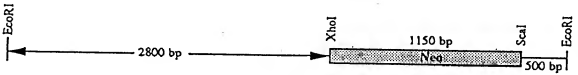


Figure 1

484

Stop 4L

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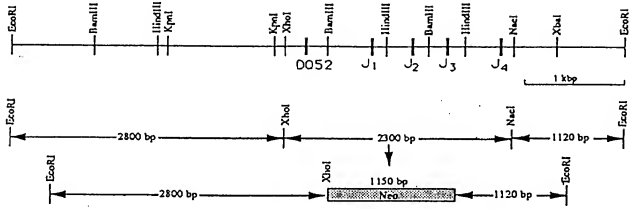
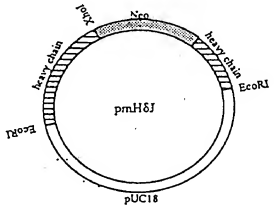
(A) Targeted mouse heavy chain J genes

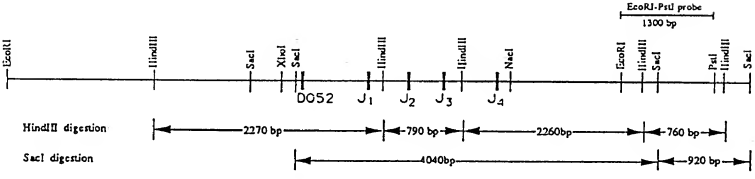


(B) Inactivation vector pmHδJ



(C) Southern analysis of pmHδJ-targeted ES colonies

Wild type ES cell genome



Targeted ES cell genome

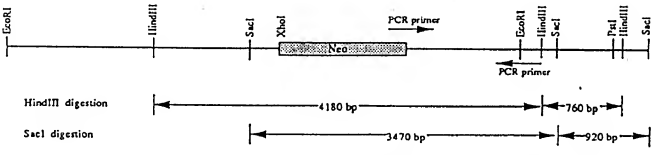


Figure 2

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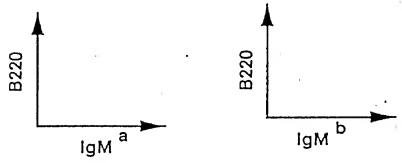
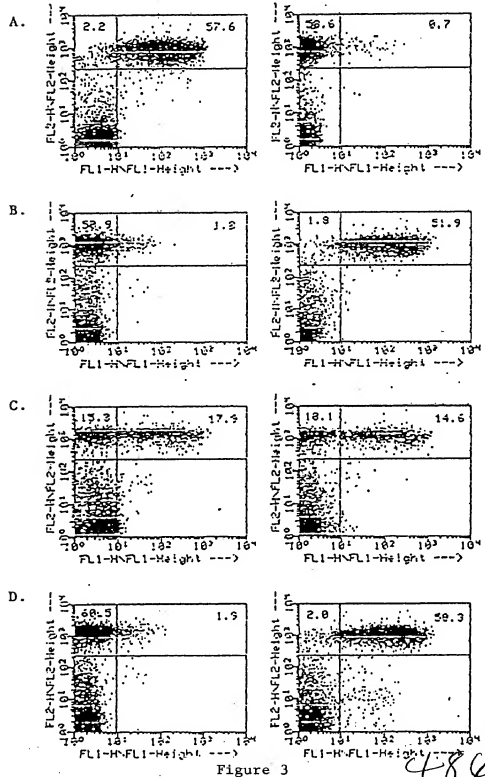
J_H deletion blocks cell surface IgM expression





a allotype

b allotype

a / b F1

ΔJ_H / b F1

Figure 3

4849

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Staining of peripheral blood lymphocytes with fluorescent anti-a allotype (A, D), anti-b allotype (B,E) or anti-B220 (C, F). (A, B, C) JH-deletion homozygous mutant mouse 244-3-2/F2-7, (D) A allotype control mouse, (E) B allotype control (F) control mouse. The number in each panel indicates the percentage of cells stained with the specific antibody.

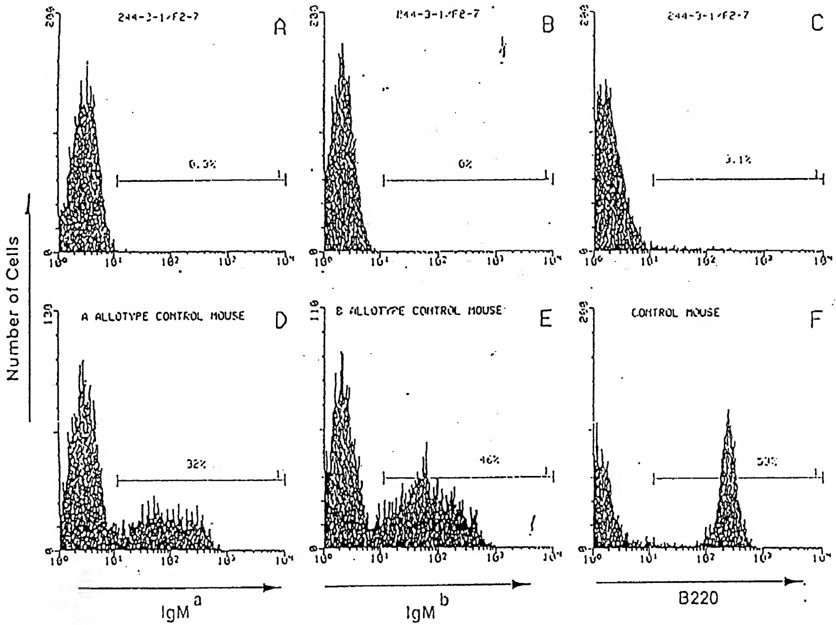

Figure 4

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INACTIVATION OF KAPPA CONSTANT REGION

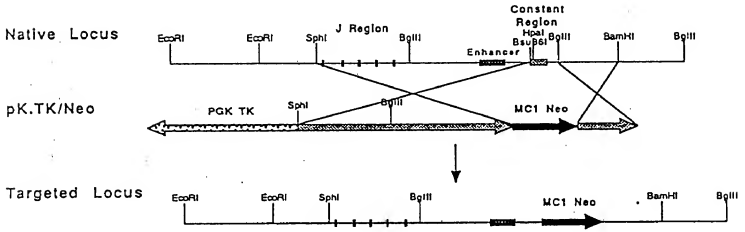

Figure 5

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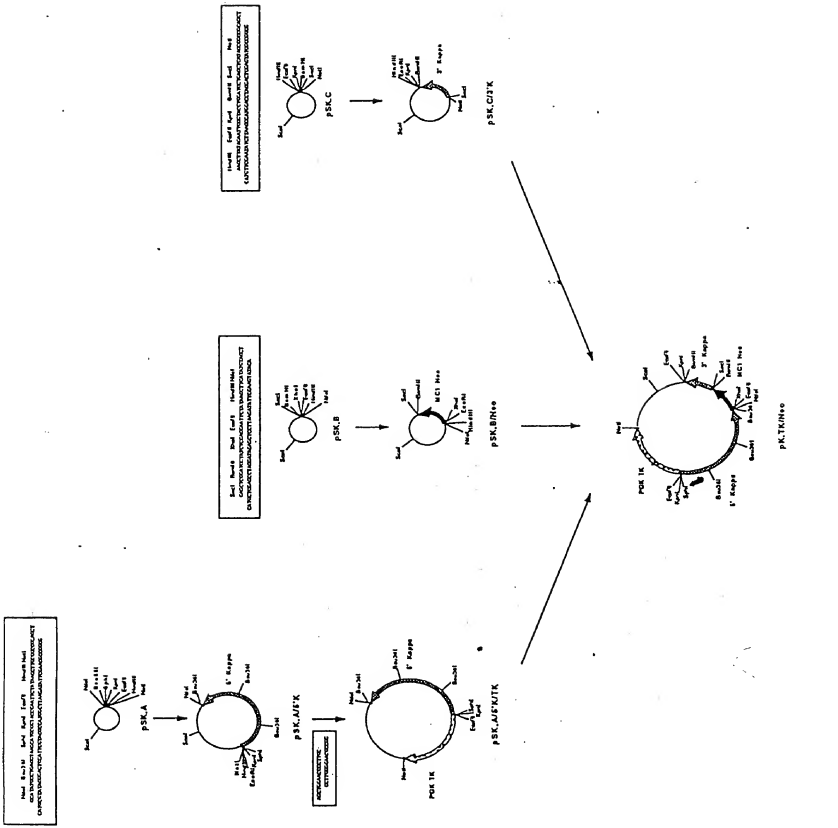

Figure 6

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SOUTHERN ANALYSIS OF LIGHT CHAIN Cκ-TARGETED E14-1 CELLS

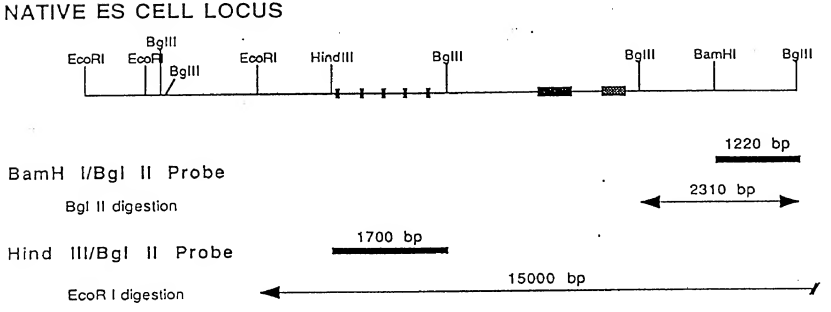
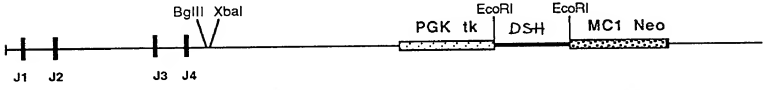


Figure 7

490

KAPPA J/CONSTANT REGION INACTIVATION

J REGION KNOCKOUT VECTOR



TARGETING SCHEME

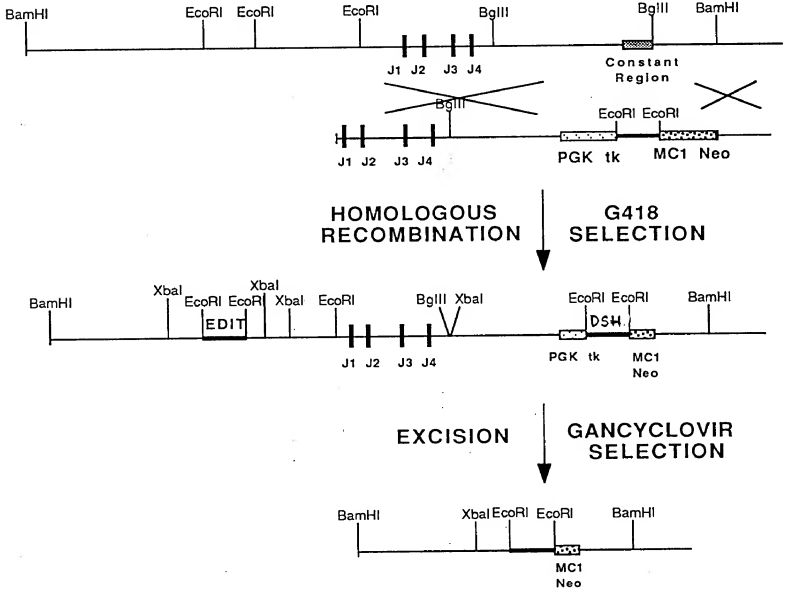


Figure 8

491

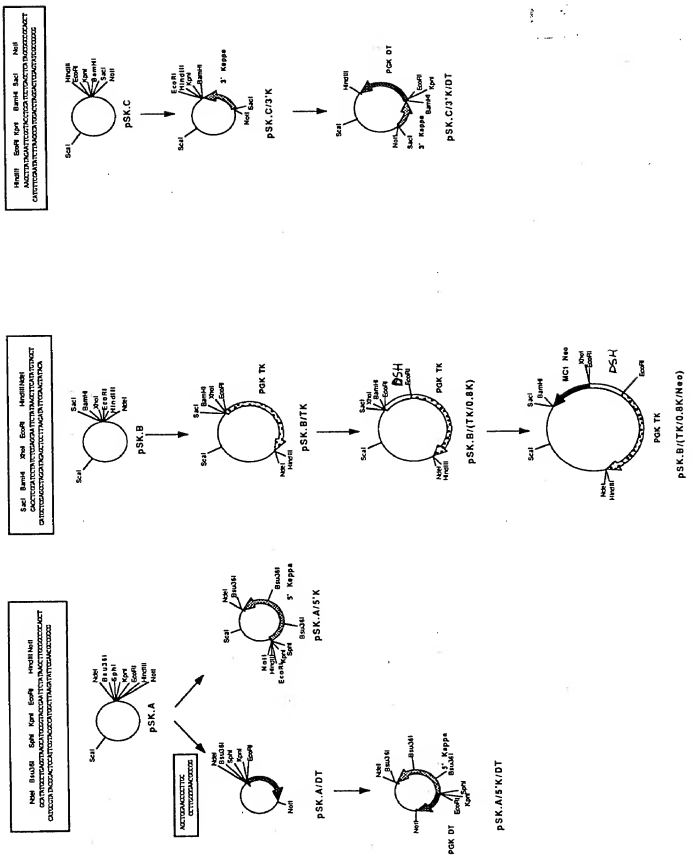


Figure 9

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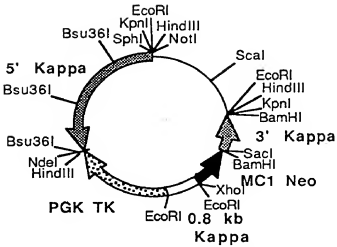
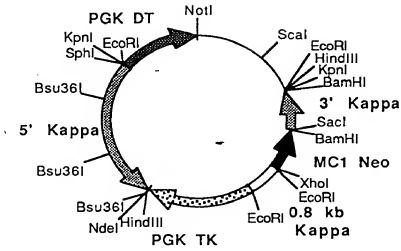
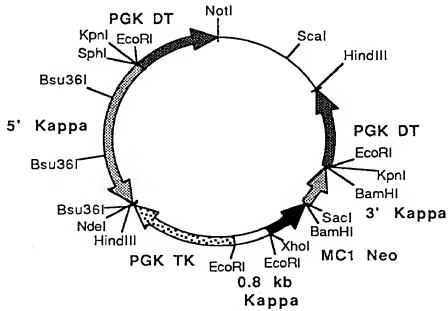
KAPPA J/CONSTANT REGION DELETION VECTORS



pK.(TK/0.8K/Neo)



pK.DT/(TK/0.8K/Neo)



pK.DT/(TK/0.8K/Neo)/DT

Figure 10

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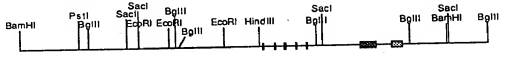
SOUTHERN ANALYSIS OF LIGHT CHAIN Jκ/Cκ-DELETED E14-1 CELLS

NATIVE ES CELL LOCUS

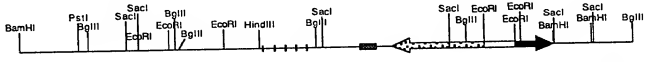


BamH I/Bgl II Probe
Bgl II digestion — 1220 bp

Hind III/Bgl II Probe
EcoR I digestion — 2310 bp

EcoR I Probe
BamH I digestion — 1700 bp, 15000 bp
Bgl II digestion — 880 bp
Sac I digestion — 2570 bp, 5490 bp

Cκ-TARGETED ES CELL LOCUS

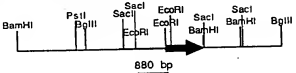


BamH I/Bgl II Probe
Bgl II digestion — 1220 bp, 4890 bp

Hind III/Bgl II Probe
EcoR I digestion — 1700 bp, 7760 bp

EcoR I Probe
BamH I digestion — 880 bp, 880 bp
Bgl II digestion — 2570 bp, 4890 bp
Sac I digestion — 5490 bp, 3080 bp

JκCκ-DELETED ES CELL LOCUS



EcoR I Probe
880 bp

BamH I digestion — 5530 bp
Bgl II digestion — 5820 bp
Sac I digestion — 2040 bp

Figure 11

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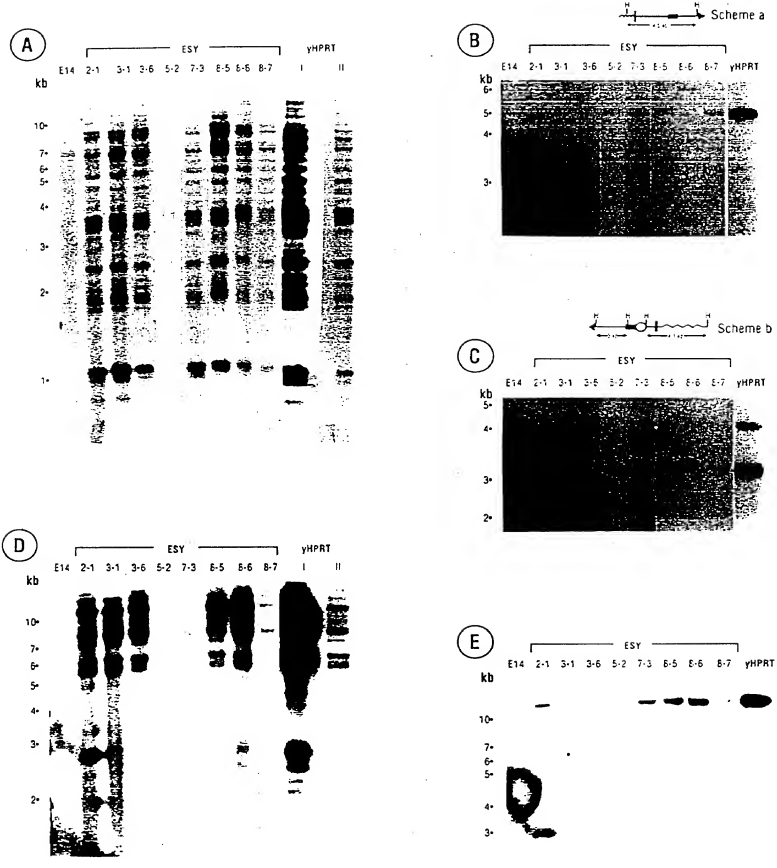

Figure 12

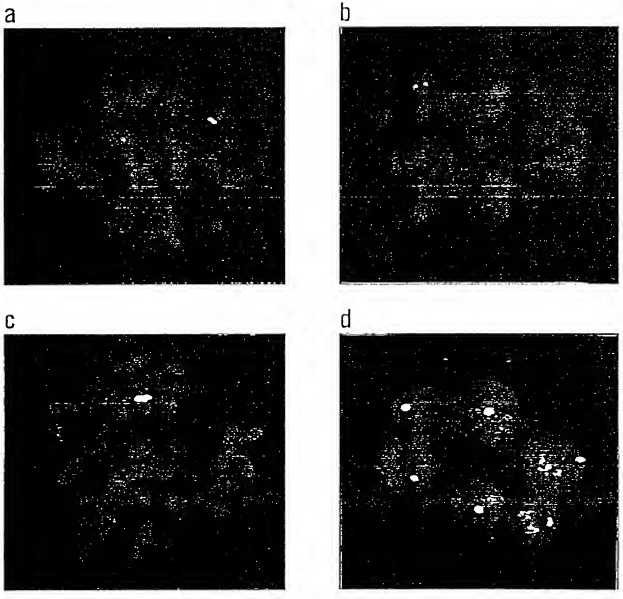

Figure 13

4 96

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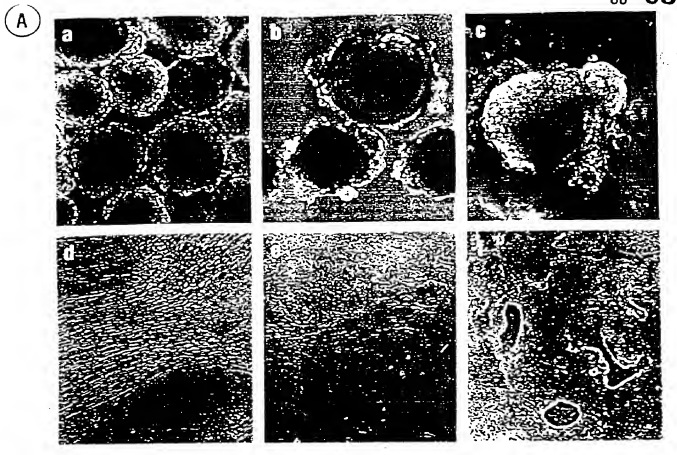

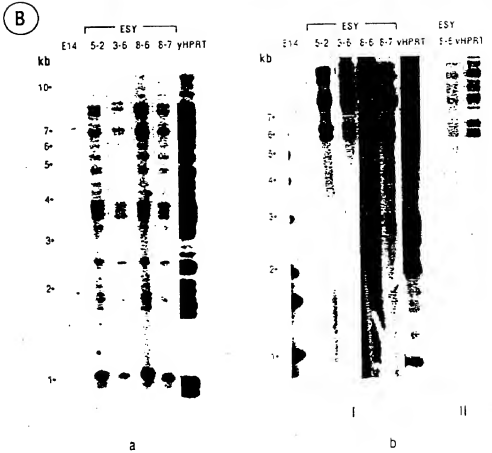

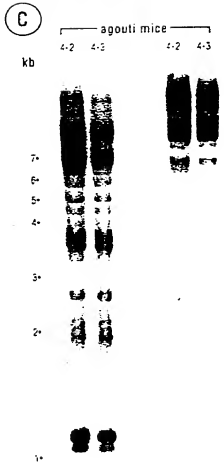

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Figure 14

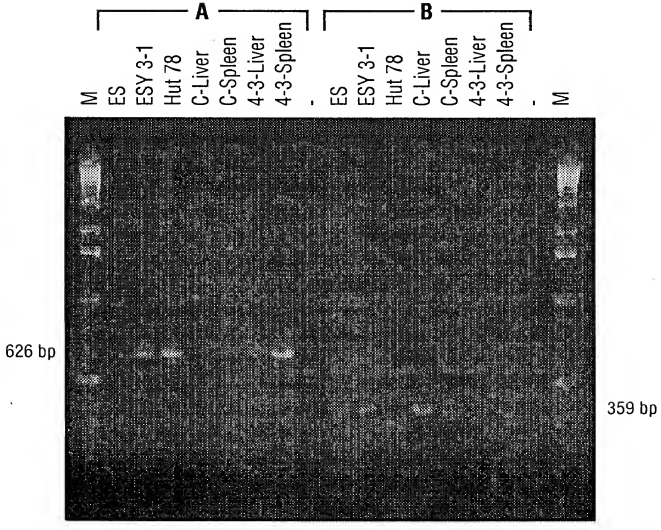

Figure 15

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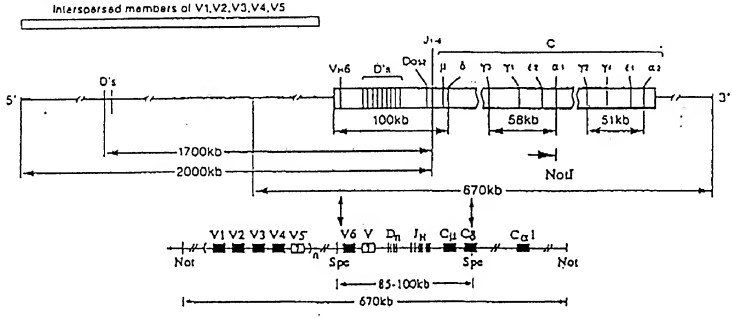
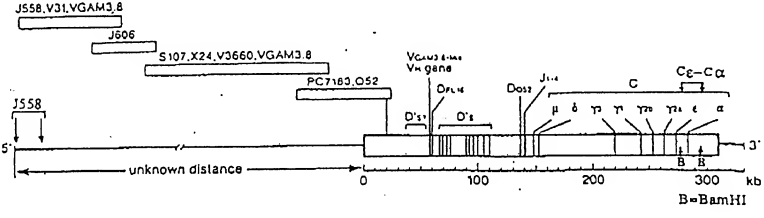
(A) Human heavy chain locus



(B) Mouse heavy chain locus



(C) Human heavy chain replacement YAC vector

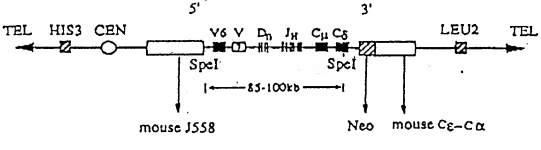


Figure 16

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Mouse Breeding Scheme

Cross IA.

heterozygous inactive Murine IgH
X
heterozygous inactive Murine IgK

MIgH (inactive) MIgK
MIgH MIgK
X
MIgH MIgK (inactive)
MIgH MIgK

↓

F1 (cross I A)

MIgH (inactive) MIgK (inactive)
MIgH MIgK

Cross I B.

heterozygous Human IgH
X
heterozygous Human IgK

MIgH MIgK HIgH
MIgH MIgK
X
MIgH MIgK HIgK
MIgH MIgK

↓

F1 (cross I B)

MIgH MIgK HIgH HIgK
MIgH MIgK

Cross II. F1 (cross I A) x F1 (cross I B)

↓

F2 Quadruple Heterozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH MIgK

Cross III. Intercross F2 mice

↓

F3 DOUBLE Homozygotes

MIgH (inactive) MIgK (inactive) HIgH HIgK
MIgH (inactive) MIgK (inactive)

Figure 17

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MAMMALIAN HOST GENOTYPES

	Hetero- or Hemi-zygous Mice	Intercross Product Mice*
I.	ΔmIgL mIgH mIgL mIgH	ΔmIgL mIgH ΔmIgL mIgH
II.	mIgL ΔmIgH mIgL mIgH	mIgL ΔmIgH mIgL ΔmIgH
III.	mIgL mIgH hIgH mIgL mIgH	mIgL mIgH hIgH mIgL mIgH hIgH
IV.	mIgL mIgH hIgL mIgL mIgH	mIgL mIgH hIgL mIgL mIgH hIgL
V.	Animal I X Animal II	
	ΔmIgL mIgH mIgL ΔmIgH	ΔmIgL ΔmIgH ΔmIgL ΔmIgH
VI.	Animal III X Animal V	
	mIgL mIgH hIgH ΔmIgL ΔmIgh	ΔmIgL ΔmIgH hIgH and ΔmIgL ΔmIgH hIgH ΔmIgL ΔmIgH hIgH ΔmIgL ΔmIgH
VII.	Animal IV X Animal V	
	mIgL mIgH hIgL ΔmIgL ΔmIgH	ΔmIgL ΔmIgH hIgL and ΔmIgL ΔmIgH hIgL ΔmIgL ΔmIgH hIgL ΔmIgL ΔmIgH
VIII.	Animal VI X Anmial VII	
	ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH	ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH hIgL hIgH
	mIgL mIgH hIgL hIgH ΔmIgL ΔmIgH	ΔmIgL ΔmIgH hIgL hIgH and ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH hIgL hIgH ΔmIgL ΔmIgH
IX.	Animal III X Animal IV	
	mIgL mIgH hIgL hIgH mIgL mIgH	mIgL mIgH hIgL hIgH mIgL mIgH hIgL hIgH
X.	Animal II X Animal IX	
	mIgL ΔmIgH hIgL hIgH mIgL mIgH	mIgL ΔmIgH hIgL hIgH and mIgL ΔmIgH hIgL hIgH mIgL ΔmIgH hIgL hIgH mIgL ΔmIgH
XI.	Animal I X Animal IX	
	ΔmIgL mIgH hIgL hIgH mIgL mIgH	ΔmIgL mIgH hIgL hIgH and ΔmIgL mIgH hIgL hIgH ΔmIgL mIgH hIgL hIgH ΔmIgL mIgH

*Not all possible genotypes from intercrosses are shown.

Δ = functionally inactive locus
m = mouse endogenous gene
h = human transgene
IgH = immunoglobulin heavy chain
IgL = immunoglobulin light chain

FIGURE 18

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